

## Original Research Article

### ***In-vitro* Evaluation of Polyherbal Formulation in the Development of Carious Lesions in Deciduous Teeth**

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**Abstract:** The study was conducted to evaluate the effect of natural antioxidants in the development of caries in the enamel of deciduous teeth. The herbal formulation contains antioxidant potential herbs which were formulated as tooth paste. 24 deciduous teeth were selected for the study and divided into 2 groups. Acid-resistant varnish was applied on the teeth, leaving only one area of 5 mm x 1 mm of dental enamel exposed. Group I served as control applied with tooth paste base, and group II was served as test applied with herbal formulation paste. The tested products were applied on the teeth according to the manufacturer's recommendations and the teeth were stored in a moist environment for 24 hours. Each group of teeth was then subjected to a pH cycling model for 14 days, after which the teeth were cut through the center for an analysis of the depth of the carious lesion by polarized light microscopy. Comparisons were made between the control and herbal formulation paste group. There was significant ( $P < 0.001$ ) decrease in the lesion with the herbal formulation loaded tooth paste applied group compared to control group. The anti-caries activity may be due to its antioxidant activity of herbs present in the herbal formulation.

**Keywords:** Antioxidant, Herbal Formulation, Anti-Caries and Deciduous Teeth

#### **INTRODUCTION**

Dental caries continues to be one of the most prevalent chronic diseases worldwide. Dental caries, or "tooth decay" as it is more colloquially known, is a microbiologic disease [1] that has implications both local to the oral cavity and in extreme cases, systemically. Globally, 60-90% of children and nearly 100% of adults have teeth affected by dental caries [2]. The first clinical sign of this demineralization is a slight color change in the enamel surface – the initial caries lesion; if oral pH is restored to a more neutral level by the removal of bacterial plaque and/or carbohydrates, then available calcium, phosphate, and hydroxyl ions in the saliva and plaque participate in re-uptake into the enamel crystalline structure [3]. Thus the dental caries is thought of a chronic and on-going disease, with the pendulum swinging between demineralization of tooth structure during periods of low pH and remineralization during periods of neutral/higher pH. When the teeth are allowed to demineralize for extended periods of low pH, the eventual total mineral loss is too much to overcome by remineralization and the enamel sub-structure collapses, forming a cavity [4].

Recently, it has been claimed that the imbalances in levels of free radicals, reactive oxygen species, and antioxidants in saliva play an important role in the onset and development of dental caries [5]. Antioxidants are molecules that inhibit oxidation of other molecules, thereby preventing formation of free radicals. These free radicals can cause harmful chain reactions that are responsible for cell damage or cell death, which in turn lead to carcinogenesis. Antioxidants neutralize these radicals by donating their electrons thereby ending the electron taking reaction [6]. Antioxidants change the progress of oral problems such as periodontitis, gingivitis by compromising antioxidant capacity of crevicular fluid and plasma. Cranberries were capable of having antibacterial activity against *Streptococcus mutans* and stopping dental caries by its free radical scavenging activity [7].

In bracket bonding, to increase bond strength values ascorbic acid solutions that were hard to prepare were used. For maxillary expansion, there are several studies researching the effect of antioxidant agents on bone formation or maturation. The effect of antioxidants on bone formation was statistically significant by using vitamin C [8]. Treating peri-

implantitis involves antioxidant supplementation that grape seed extract has positive effect on treating peri-implantitis [9]. The aim of the present study was to investigate the effect of natural antioxidants in the development of caries in the enamel of deciduous teeth.

**MATERIALS AND METHODS**

**Tooth Paste Base**

The standard tooth paste was formulated as per the method of Sharma, 1998 [10]. The following ingredients were properly mixed to get tooth paste base. Calcium Carbonate 3.5 gm (Abrasive), Sodium Lauryl Sulphate 0.15 gm (Surfactant), Glycerin 3.0 gm (Anticrusting agent), Methyl cellulose 0.1 gm (Gelling agent), Sodium Saccharine 0.03 gm (Sweetener), Methyl paraben 0.01 gm (Preservative), Propyl paraben 0.002 gm (Preservative), Titanium dioxide 0.05 gm (Opacifier), Menthol 0.015 gm (Flavoring agent) and sufficient Purified water (Vehicle).

**Composition of Antioxidant Herbal Preparation [11]**

Each gram of herbal preparation contains powders of *Aegle marmelos* Corr. (Rutaceae; fruit, 150 mg), *Elettaria cardamomum* Maton. (Zingiberaceae; seeds, 125 mg), *Glycyrrhiza glabra* L. (Papilionaceae; root, 150 mg), *Citrus aurantifolia* Swingle. (Rutaceae; Fruits, 150 mg), *Rosa damascena* Mill. (Rosaceae; flower petals, 150 mg), *Cissus quadrangularis* Linn. (Vitaceae; Whole Plant, 150 mg) and *Saccharum officinarum* Linn (Poaceae; root, 125 mg). The 3 gms of Antioxidant herbal preparation was mixed with the tooth paste base and used for the study.

**In vitro Anti-Caries Study [12]**

Twenty four caries-free deciduous teeth were selected, devoid of stains or any other defects visible under a stereoscopic magnifying glass. The teeth were supplied by the tooth bank of Dentistry department, Sri Lakshmi Narayana Institute of Dental Sciences. After their selection, the teeth were immersed in 0.1% thymol and stored in a refrigerator until their use. Before using the teeth, they were cleaned with pumice and water, after which they were distributed into two groups of 12 each. Group I served as control applied with toothpaste base and group II as test applied with herbal

formulation. A layer of acid resistant varnish was applied on all the faces of the teeth, leaving only one 5 x 1 mm area of enamel exposed. The control and test tooth paste formulation were applied and stored for 24 hours in a moist environment.

This storage period was followed by prophylaxis using pumice stones and deionized water, after which the groups were subjected to 10 pH cycles for 14 days. To this end, the teeth were immersed for 3 hours in a demineralizing solution (2.0 mM of calcium and phosphate in 75 mM acetate buffer at pH 4.3) at a temperature of 37°C. The teeth were then washed in deionized water, dried with paper towels and placed in a container with a remineralizing solution (calcium 1.5 mM/L, phosphate 0.9 mM/L, potassium chloride 150 mM/L, and cacodylate buffer 20 mM/ L, pH 7.0), where they were left for 21 hours. Before and after immersion in the demineralizing and remineralizing solutions, both groups were treated with the control and herbal formulations. The de- and remineralizing solutions were changed daily to prevent depletion or saturation of the solution and accumulation of enamel dissolution products. After the pH cycles, the teeth were cut with a double-faced diamond disk coupled to a cutting machine and prepared for analysis by polarized light microscopy to determine the presence or absence of carious lesions and the depth of the lesions.

**Statistical Analysis**

Comparisons were made between the control and test groups. The data were analyzed statistically by un-paired ‘t’ test with a 5% level of significance.

**RESULT**

The effect of herbal formulation on *in vitro* development of carious lesions in deciduous teeth was shown on the table I. The average depth of the lesion in the teeth applied with the tooth paste base and herbal formulation loaded tooth paste were 320.02 ± 3.52 and 148.15 ± 3.94 respectively. There was significant (P<0.001) decrease in the lesion with the herbal formulation loaded tooth paste applied group. The percentage reduction in the lesion depth of herbal formulation applied group was 53.71%.

**Table-I: Effect of Herbal Formulation on *in vitro* development of carious lesions in deciduous teeth**

Group	Average Depth (µm) of the Lesion	% Reduction of Lesion Depth
Control	320.02 ± 3.52	-
Herbal Formulation	148.15 ± 3.94***	53.71

Data’s were expressed as Mean ± SEM (n=6)  
 \*P<0.05, \*\* P<0.01 and \*\*\* P<0.001 Vs Control

**CONCLUSION**

The study was to investigate the *in vitro* effect of natural antioxidants in the development of caries in the enamel of deciduous teeth. The natural herbal formulation containing tooth paste protected the formation of dental caries by decreasing the depth of

induced caries lesion in teeth. The result shows that, anti-caries activity of herbal formulation may b due to its rich antioxidant which prevents the formation of free radical generations. Further study may be focused on *in vivo* and the isolation of active principle responsible for its action.

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